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ABSTRACT

This paper discusses the risk assessment associated with microbiology instruction based on grade level, general control measures, appropriate activities for middle school and high school students, the preparation and sterilization of equipment, and safe handling techniques. Appended are instructions and figures on making wire loops and the standard operating procedure for preparing nutrient agar plates. (YDS)

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MICROBIOLOGY – Safety Considerations

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CONASTA 50
Workshop

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1. SAFE TECHNIQUES IN MICROBIOLOGY

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1.1 DEFINITION OF MICROBIOLOGY

Microbiology is the study of microscopic organisms including bacteria, fungi (moulds and yeast), viruses, microscopic algae and protozoa.

1.1.1 GENERAL HAZARDS

Exposure to microorganisms may occur through contact with skin, eyes, puncture wounds, inhalation, or ingestion. Exposure may involve individual staff or students using the microorganisms, others that use the classroom or work area, cleaners or the wider community. The outcome following exposure depend on the microorganism involved, the exposure level and the route of exposure. For example *Staphylococcus aureus* is a bacterium found on the skin of 30-50 % of people. If it penetrates the skin it may give rise to septic pimples and boils. It may also cause Staphylococcal pneumonia if it infects the lungs and Staphylococcal meningitis if it infects the cerebro-spinal fluid.

1.1.2 RISK ASSESSMENT

Whenever microbiology is studied there are potential risks from the hazards of infection. It is the strict observance of correct procedures, which enables students and staff to work safely with microorganisms. This includes selecting the appropriate level of practical activity:

* for the age group.
* for the available facilities
* and for the level of training and experience of the teacher and the laboratory officer.

Standard operating procedures should be documented for all activities undertaken and these should be strictly adhered to. The standard procedures should be the final product following the normal OHSW risk assessment and should use the safest practicable methods for the activity you wish to do, with the facilities you have. Procedures should ensure that contamination does not occur to the samples, to the air, to the laboratory, to oneself, to other people or to the wider community and the environment. These procedures should also include the equipment and facilities required in, preparation, experimental techniques, cleanup and disposal as well as emergency procedures for spills and first aid.

The following information is designed to indicate the various risks and ways to control them.

1.1.3 GENERAL CONTROL MEASURES

If class discipline can not be relied on, student experiments should not proceed.

Selection of Media: Standard nutrient broth or nutrient agar is sufficient for school culture

work. Media designed to select pathogens (e.g. blood agar, McConkey etc.) must not be used.

Selection of Bacteria: Schools should only use Risk Group 1 micro-organisms (as defined in AS2243.3)- those that pose low individual & community risks. These are microorganisms that are unlikely to cause human, plant or animal disease.

The following list includes suggested "safe" microorganisms. Schools should restrict themselves to this list, and obtain them exclusively from reputable suppliers.

Bacteria

Acetobacter aceti
Agrobacterium tumefaciens
Bacillus subtilis
Chromobacterium lividum
Chromatium species
Erwina carotovora (=E. atrospetica)
Escherichia coli *
Lactobacillus species
Micrococcus luteus (=Sarcina lutea)
Photobacteium phosphoreum
*Pseudomonas fluorescens***
Rhizobium leguminosarum
Rhodopseudomonas palustris
Spirillum serpens
*Staphylococcus albus (epidermidis)***
Streptococcus lactis
Streptomyces griseus
Vibrio natriegens (=Beneckea natriegens)

Viruses

Bacteriophage (T type) (host E. coli)
Cucumber Mosaic Virus
Potato Virus X
Potato Virus Y (Not the virulent strain)
Tobacco Mosaic Virus
Turnip Mosaic Virus

Fungi

Agaricus bisporus
Armillaria mellea
Botritis cinerea
Botrytis fabae
Chaetomium globosum
Coprinus lagopus
Fusarium solani
Fusarium oxysporum
Helminthosporium avenae
Mucor hiemalis
Mucor mucedo
Myrothecium verucaria
Penicillium roqueforti
Phycomyces blakesleanus
Physalospora obtusata
Phytophthora infestans
Pythium debaryanum
Rhizopus sexualis
Rhizopus stolonifer
Rhytisma acerinum
Saccharomyces cervisiae
Saccharomyces ellipsoides
Saprolegina litoralis
Schizosaccharomyces pombe
Sclerotinia fructigena
Sordaria firmicola
Sporobolomyces species

* *Some strains have been associated with health risks. Reputable suppliers will ensure that acceptable strains are provided.*
** *These organisms have been known to infect debilitated individuals and those taking immunosuppressive drugs.*

List taken from: Department of Education and Science, 1985, *Microbiology: An HMI Guide for Schools and Non-advanced Further Education*, Her Majesty's Stationery Office, London, pg 20.

The following microorganisms have previously been used in schools but are no longer considered appropriate. These should not be used in schools.

Aspergillus nidulans
Aspergillus niger
Chromobacterium violaceum
Clostridium perfringens (welchii)
Penicillium chrysogenum
Penicillium notatum
Pseudomonas aeruginosa
Pseudomonas solanacearum
Pseudomonas tabacci
Serratia marcescens
Staphylococcus aureus
Xanthomonas phaseoli

List taken from: Department of Education and Science, 1985, *Microbiology: An HMI Guide for Schools and Non-advanced Further Education*, Her Majesty's Stationery Office, London, pg 21.

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Potential Pathogens: All microorganisms should be treated as potential pathogens. Even microorganisms not normally associated with human diseases, are opportunistic pathogens and may cause infection in the young, the aged and in immunodeficient or immunosupressed individuals. In addition, samples may become contaminated or may mutate.

Size Limitations: Cultures should be kept to the minimum size and number required to do the job.

Incubation Temperature: The incubation temperature should be restricted to an upper limit of $30^{0}C$ to reduce the danger of isolating pathogens adapted to human body temperature. Anaerobic conditions (ie. devoid of oxygen) should also be avoided for the same reason.

Aerosols: Aerosols (suspended particles of liquid containing microorganisms) can easily contaminate the laboratory if procedures are poor. Care must be taken when flaming loops, opening bottles, using pipettes and any other procedure that may produce aerosols. Spills may also cause aerosols. (see section 1.1.9 Spills and First Aid)

Culturing from the Environment: If microorganisms are cultured from the environment care must be taken as they may isolate pathogens. These petri dishes should not be opened following inoculation and they should never be sub-cultured. Samples should never be taken from areas likely to harbor pathogens, eg toilets, bird or animal cages, dead animals and faecal material. Soil cultures may also isolate Tetanus bacteria, and should not be attempted.

Extra Laboratory Rules for Microbiology:

* No eating, drinking or storing of food for human consumption in the laboratory.
* Any activities that introduce objects into the mouth must also be prevented, eg licking labels, sucking pencils and pipetting by mouth.
* Staff & students should wear laboratory coats/aprons while in the laboratory. These coats/aprons must never be worn outside the laboratory eg. the lunchroom, toilets etc.
* All cuts or broken skin must be covered by a water resistant dressing eg a Band-Aid and gloves.
* Benches should be clear of all non-essential materials including books and notes.
* When all practical activities are finished, benches should be wiped with a suitable disinfectant eg. 0.1% solution of sodium hypochlorite, or 70% alcohol.
* Hands should be washed with soap and water after removing gloves, following cleanup of a spill and before leaving the laboratory. (See section 1.1.10 for Correct Hand Washing Techniques)

1.1.4 APPROPRIATE ACTIVITIES FOR VARIOUS YEAR LEVELS

Junior School - Year 6-7

Only microorganisms with little, if any, known risk should be used. e.g. observation of moulds, yeasts and algae. These should be observed in closed containers that the students can not open.

No special training is required for the teacher or laboratory officer.

Middle School - Year 8-10

At this level, culturing techniques with known organisms from reputable suppliers and the culturing of organisms from the environment is acceptable. All petri dishes must be sealed following inoculation & during examination. Samples should not be taken from environments likely to contain organisms harmful to humans eg. body surfaces, coughs, sneezes, nasal discharge etc.

At this level, both the teacher & the laboratory officer require a working knowledge of aseptic technique, safe disposal technique and must be able to recognise contamination. This level of knowledge would be acquired from a short in-service course in microbiology.

Senior School - Year 11-12

At this level, sub-culturing and transfer procedures using known organisms are appropriate. In addition, culturing of organisms from the environment including body surfaces but without opening of petri dishes following inoculation is acceptable.

At this level, both the teacher & the laboratory officer require good aseptic technique. This can only be provided through training & practice extending beyond the level of a short course. Work should not be carried out by non-specialised staff or if appropriate facilities are not provided.

1.1.5 APPROPRIATE FACILITIES

No activity should be undertaken unless appropriate facilities are available. Facilities required depend on the activity undertaken and disposal methods used.

Three main areas need to be considered:
* The preparation room where media and equipment are prepared and sterilised.
* The classroom where experimental procedure will occur.
* The methods for the disinfection or sterilisation of equipment prior to disposal or reuse.

Bacteria from Risk Group 1 require no special containment equipment but facilities and work practices should conform to Level 1 Containment (as defined in AS2243.3). The minimum requirements include:
* adequate space for the number of people in the laboratory.
* easily cleaned surfaces.
* wash basin with hot and cold water, preferably near the exit.
* an autoclave for decontamination of infectious waste.

1.1.6 PREPARATION & STERILISATION OF EQUIPMENT

Generally, preparation and sterilisation procedures are carried out by the laboratory officer in the preparation room.

It is important to distinguish between sterilisation (killing of all microbiological life including bacterial and fungal spores) and disinfecting (likely to kill all but the most resistant microorganisms such as *Tetanus* bacterial spores). Within the school environment only a correctly maintained and operated autoclave is capable of sterilisation. All other methods, including pressure cooker, microwave and chemical means can only be considered disinfecting. This is generally adequate for a school's requirement in microbiology.

Disinfectants: Disinfectants and antiseptics (disinfectants for use on living surfaces e.g. skin) vary in their ability to kill bacterial, viruses, fungi, spores and protozoa.

Disinfectants should always be diluted and used according to the manufacturer's instructions. The Material Safety Data Sheet should also be consulted for specific protective equipment and ventilation requirements.

The following types of disinfectants are suitable for use in schools.

Alcohols have good activity on bacteria, and fungi but less on viruses and poor activity on spores. 70% ethanol is rapid acting and dries quickly. 90% ethanol if good for viruses. 100% ethanol is NOT an effective disinfectant. 60-70% Isopropyl Alcohol (Propan-2-ol) is also effective.

Chlorhexidine has good activity on gram-positive bacteria but less activity on gram-negative bacteria, viruses and fungi and poor activity on spores. It has low toxicity and irritancy and so is a good antiseptic. 0.5% for face - 4% for other skin. It is often combined with alcohol, which may dry the skin.

Hypochlorite has good activity on bacteria, fungi and viruses, but less activity on spores. Varying amounts of available chlorine in hypochlorite solutions are required for different purposes. They must be prepared fresh daily from the concentrated stock solution to ensure the correct level of available chlorine. 1% for spills, 0.25% for discard jars, 0.1% for cleaning benches and 0.05-0.1% for equipment and instruments.

Providone-Iodine as 10% aqueous or alcoholic solutions are also suitable skin disinfectants but they stain.

Quaternary Ammonia compounds are not effective disinfectants against many bacteria and viruses.

Peracetic acid, aldehydes and phenolic disinfectants are considered too hazardous for use in schools.

Autoclave: Autoclaves must be registered with DAIS according to OHSW Regulation 3.4.3 (1), and (7) requires that autoclaves are inspected and certified as

safe to use, by a competent person (eg a boiler & pressure vessel inspector) each year.

Autoclaves can be used to sterilise equipment and media prior to use. In general, items should be sterilised at 121 ^{O}C and 15 p.s.i. (or 103kNm^{-2}) for 15 minutes. The Manufacturer's instruction manual should be followed carefully. For safety reasons and to ensure that items are correctly sterilised, only trained operators should operate the autoclave. AS4187 should be referred to for procedures to ensure correct steriliation of equipment.

A few points to remember:
* Do not wrap or cover items with foil as this impedes the steam penetration. Kraft paper or special autoclave bags available from medical suppliers are more appropriate.
* All air must be driven off before the pressure valve is closed or the correct temperature and pressure will not be reached, and items will not be sterile.
* Autoclaves should not be opened until the pressure has returned to zero and the contents have been allowed to cool.
* extreme care must be taken when sterilising liquids, the exhaust valve should not be opened quickly or bottles may explode.

Pressure Cookers: Pressure cookers can be used if an autoclave is unavailable. (see limitations above) The pressure cooker should have a pressure valve to ensure items are sterilised, at the correct temperature and pressure for the correct length of time. The manufacturer's instructions should be followed carefully. Items are sterilised in the same way as when using an autoclave.

Pipettes: Pasteur pipettes should be plugged with non-absorbent cotton wool and any protruding fibres burnt off. They are then wrapped in brown paper (eg Kraft) or foil, alternatively they can be placed in pipette canisters or test tubes and the ends of the test tubes sealed with paper or foil and masking tape. They can then be sterilised in a dry heat oven at 160 ^{O}C for at least 1 hour, allowing adequate time to reach the correct temperature. Temperatures over 170 ^{O}C will char brown paper and cottonwool.

Similarly graduated pipettes may be plugged, wrapped and sterilised.

Following use pipettes should be immediately immersed in a container of disinfectant and sterilised before disposal or washing. (A 600 ml tall beaker or a 2L plastic measuring cylinder makes a good discard jar.)

Swabs: Cottonbuds can be wrapped in brown paper and autoclaved or wrapped in foil/ brown paper and dry heat sterilised as above. Some cottonbuds are treated with anti-microbial agents and it is usually more reliable to use hospital grade swabs, available from medical supply companies.

Following use, swabs should be immersed in a container of disinfectant and sterilised before disposal.

Test Tubes & Other Glassware: Test tubes and other glassware should be plugged with cotton wool and capped with foil or brown paper, and then sterilised by dry heat as above. McCartney bottles and Schott bottles do not require cottonwool plugs and should just have their lids screwed on firmly and then released a quarter turn, prior to sterilisation.

Once used, glassware should either be placed in disinfectant or autoclaved prior to cleaning.

Loops: Wire loops for transfer of bacteria can be made from 24 swg nichrome wire held in chuck needle holders or glass tubing that has had the wire inserted and the neck sealed closed by the application of heat.

Loops are sterilised by heating to red hot in a bunsen flame. Flame heating loops carrying a culture may splatter and produce aerosols to avoid this, loops should be drawn slowly from the cooler to the hotter part of the flame. They should also be cooled before use (e. g. on a vacant part of the agar plate) to prevent killing the bacteria that you wish to culture. Alternatively the loop may be dipped in 70% alcohol and then flamed.

Loops are sterilised just prior to and immediately following use.

Spreaders & Forceps: Spreaders are L-shaped glass rods used to spread a liquid culture evenly over a petri dish. Spreaders and forceps are sterilised by dipping them in alcohol and lighting the alcohol in a bunsen flame. The alcohol is allowed to burn off before use. Spreaders may break if held in the bunsen for too long.

Spreaders and forceps are sterilised just prior to and immediately after use.

Preparation of Agar Plates: Following the instruction on the jar, the powdered agar media is added to water in a large conical flask. It is heated until the agar dissolves at 95 ^{O}C. (This is best done in a water bath e.g. a saucepan of water on a hotplate. Alternatively be sure to mix the contents well or uneven heating may crack the flask.) The agar is poured into Schott bottles and the lid closed firmly then released a quarter turn. Alternatively the agar may be poured into conical flasks and plug with cottonwool and cover with brown paper. The bottles/flasks are then sterilised in an autoclave or pressure cooker for 15 minutes at 121 ^{O}C & 15 p.s.i. (a microwave method for sterilisation is also available see below) The agar is allowed to cool to 50-55 ^{O}C. (NB: Agar will not set above 42^{O}C) Plastic disposable petri dishes are lined up on the bench, in a draft free room. NB: It is not recommended that glass petri dishes be used. Airconditioners, fans and fumecupboards must be turned off, windows and doors closed and foot traffic restricted, to reduce the chance of contamination to plates while pouring. The bottle/flask is unscrewed or unplugged and held at an angle. The lid from a petri dish is lifted just enough to allow the agar to be poured in. This is repeated for the other dishes until all the agar is used. Approximately 25 ml of agar per dish is ideal. The agar is allowed to set before repacking in plastic bags and storing in the fridge in an inverted position until required. (see Figure 1)

The alternate recipe for Nutrient Agar preparation, used in the Microwave Method,

can also be used. The reduced nutrient level may reduce some bacterial growth but has been used successfully for school based work.

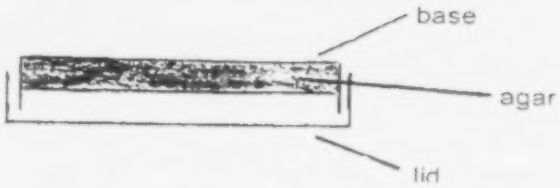

Figure 1. The Inverted Position for Agar Plates During Incubation & Storage

Prevention of Condensation: To prevent condensation problems, agar should be cooled to 50-55 ^{O}C before pouring. In addition plates may be incubated overnight at 30 ^{O}C. (this also check that the plates are sterile) If condensation is bad, sterile plates can be separated, and inverted to exclude contamination, (see figure 2) and dried in an incubator. It is inadvisable to do this once plates have been inoculated.

Figure 2 Drying Agar Plates Prior to Incubation

Microwave Method for Preparing Agar:

Taken from: Bouts, C. How I Do It - An Example of Good Practice. in "Examples of Good Laboratory Practice" Science Apparatus Committee/SASTA Laboratory Officer's Workshop. Adelaide, pp.7

Make a slurry from 12.5 gm agar , 8 gm Nutrient agar and 250 ml distilled water, in a Pyrex saucepan. Add 750 ml of boiling distilled water, stirring constantly. Heat on a hotplate again stirring constantly. When agar has dissolved the solution becomes clear. Decant into 5 X 250 ml conical flasks, plug with cottonwool and seal with cling wrap. Cook on HIGH in a microwave until they boil. Watch constantly and turn off immediately. Reduce to medium and simmer for 10 minutes. Cool and proceed as for autoclaved agar.

Preparation of Overnight Broth Cultures: Nutrient broth is prepared by adding the powder to water and stirring until dissolved, following the instructions on the jar. It is then sterilised at 121 ^{O}C & 15 p.s.i. for 15 minutes. After it is allowed to cool, a small amount of bacteria is introduced either as a solid form from a petri dish using a wire loop or as a liquid form from a broth using a wire loop or pipette. It is then incubated overnight at 30 ^{O}C.

1.1.7 SAFE HANDLING TECHNIQUES

These procedures are for use by the laboratory officer, teachers and students. Teachers should fully explain and demonstrate all techniques that the students will use, giving particular attention to safety and good aseptic technique.

Loops, Spreaders & Forceps: see above

Inoculation of Plates via Air Exposure: Petri dishes may be inoculated by exposing them to the air. When this is done the plates should be sealed and not reopened even during examination. These cultures should not be sub-cultured by anyone.

Plating Broth Cultures Using Wire Loops: The lid/plug of the culture bottle/flask is held in the little finger. (never placed on the bench) The bottle's neck is then passed through a bunsen flame. (See Figure 3) A wire loop is heated to red hot in the bunsen flame and then dipped into the broth culture carefully. Splattering may occur if the loop is not allowed to cool slightly before use. A loop full of culture is transferred to the agar plate. The loop is spread over the surface of the plate. (see Figure 4) If individual colonies are required the pattern illustrated in Figure 5 is used. The loop is then reflamed and the neck of the culture bottle is passed through the bunsen flame before the lid/plug is re-fitted.

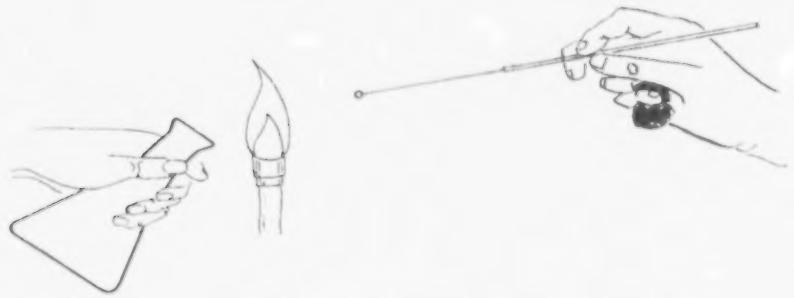

Figure 3 Flaming the Neck of a flask & Holding a Cotton Plug with the Little Fingers

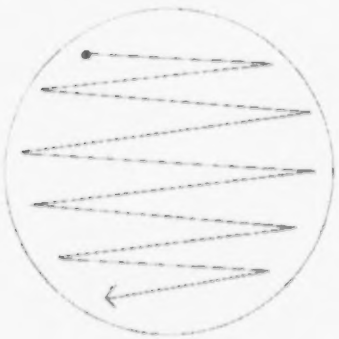

Fig 4 General Spreading Pattern

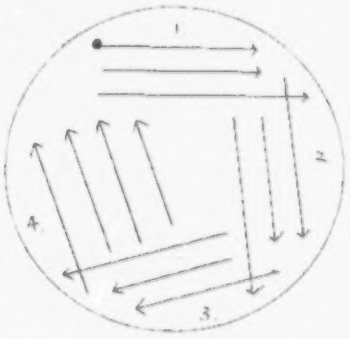

Fig. 5 Spreading Pattern for Individual Colonies

Preparing a Bacterial Lawn for Antibiotic Discs: The lid/plug of the culture bottle/flask is held in the little finger. (never placed on the bench) The bottle's neck is then passed through a bunsen flame. (See Figure 3) A pipette is partly removed from its packaging and a teat attached. 3-4 drops of a liquid culture are removed

from the culture and introduced into the petri dish. A sterile spreader is then used to spread the culture evenly over the agar. An antibiotic disc is removed from its packet with sterile forceps and carefully positioned on the agar surface using sterile forceps.

Similarly, other antiseptics or test solutions may be tested, by placing a small disc of filter paper that has been dipped into a test solution onto the surface of the agar spread with a bacterial lawn.

Taping & Incubation: After inoculation, the petri dish base and lid should be taped together as shown in Figure 6. Petri dishes should not be completely sealed as this may produce anaerobic conditions and the selection of pathogens. Plates should be labeled on their base with contents, date and student name. Ideally this should occur prior to inoculation.

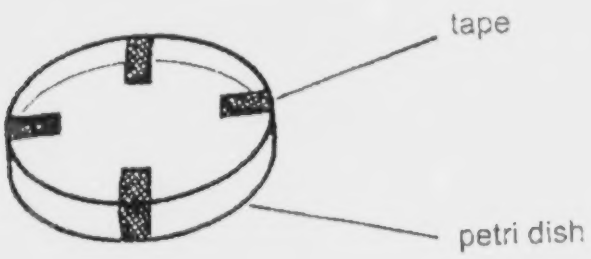

FIGURE 6 Taping of Petri Dishes Following Inoculation

Plates should be incubated in an inverted position (that is agar side upwards) to prevent condensation falling onto the colonies and contaminating them. (see Figure 1)

Plates should not be piled high otherwise they may fall spreading their contents over the bench. Dish racks or baskets should be used or the plates taped together with masking tape.

Observation of Plates: Petri dishes should be observes without opening the lid. [Yr 11-12 may open the lids but only when necessary to subculture the bacteria and only with samples of known "safe" bacteria.]

Microscopic Examination of Bacteria: This procedure should only be carried out at Year 11 – 12 level when the students have developed good aseptic techniques. Bacteria may be transferred to a microscope slide from an agar plate using a wire loop or from a broth culture with a loop or pipette. If from an agar plate the bacteria a mixed with the drop of sterile water, on the slide, using the loop. The bacteria are spread into a very thin film using the end of another slide as for blood smears. This film is then held high over a bunsen flame to dry. It must not be allowed to steam and hence produce aerosols. If the smear is not dried within a few seconds it is too thick and the bacteria will clump together and distort as they dry

and fix onto the slide. All contaminated items must be immediately placed in disinfectant. The fixed bacteria may then be stained using a Gram staining technique.

A safer method of viewing bacteria on the microscope is to use yoghurt instead of the broth culture. This is suitable for Yr 8-12

Other Practical Experiments: Other practical procedures (e.g. serial dilutions) may be attempted at Year 11-12 level when students have developed good aseptic techniques. Standard procedures found elsewhere, should be assessed and should be followed carefully.

1.1.8 Disposal and Cleanup

General Cleanup: All contaminated items should be decontaminated prior to reuse or disposal. Carefully organisation is required, to minimise re-handling and prevent the spread of contamination. Containers should be lined with autoclavable bags for collection of items to be autoclaved. (NB: Glad oven bags make a good substitute for autoclave bags)

Glassware: Items for reuse should be immediately placed in disinfectant and soaked according to the manufacturer's instructions, prior to washing. (e.g. 0.25% Sodium Hypochlorite solution, soaked overnight)

Broth Cultures: Broth cultures should be sterilised by either autoclaving or addition of a suitable disinfectant. (e.g. enough sodium hypochlorite to bring the culture to 1 % solution, left overnight) Once sterilised it may be poured down the sink.

Petri Dishes: When disposable petri dishes are no longer required they should be placed in an autoclavable bag for sterilisation before disposal. Once the bag is closed it should not be reopened to add additional plates as this may produce high level of aerosols, and hence contamination.

A pressure cooker or microwave may also be used to disinfect petri dishes prior to disposal. Only a laboratory microwave, not used for food, should be used. If none of these methods is available, you must make arrangements with a licenced prescribed waste removal company, for safe disposal. It is not permissible to discard petri dishes into general or industrial garbage without prior sterilisation.

Incubator: Following use the incubator must be thoroughly cleaned and disinfected with an appropriate disinfectant. (eg. 0.1% sodium hypochlorite)

1.1.9 Spills & First Aid

Students must report all spills to the teacher. Only the teacher or laboratory officer should be allowed to clean up such spills. If the spill is large or has caused a lot of splashing, aerosols may have been produced and the room should be evacuated for 30 minutes. When cleaning spills disposable gloves must be worn.

A spill kit should be prepared prior to starting microbiology practicals. It should include all items required to clean up a spill, including disinfectant, paper towel, gloves and plastic bags and containers for disposal.

Spills on the Body: The teacher must be informed immediately. Contaminated clothing should be removed and the affected area washed vigorously with soap and water. Medical attention may be sought if required. The incident must be documented in the first aid &/or OHSW records. Contaminated clothing must be disinfected before washing.

Cuts and Puncture Wounds from Contaminated Sharps: Immediate first aid must limit contamination to the wound and to first aid personnel. Any cut or puncture wound, caused by contaminated glass or sharps, must receive immediate medical attention.

Liquid Spills: Liquid spills should be covered with paper towel soaked in disinfectant (e.g. Sodium Hypochlorite with 1 % available chlorine) for at least 20 minutes. The paper towel should then be placed in a plastic bag for disposal. The area should be cleaned with fresh paper towels soaked in disinfectant. Large spills may require the use of spill-control pillows or similar. These must be disinfected prior to disposal.

Contaminated Broken Glassware: Contaminated broken glassware should never be picked up directly with the hands. It should be cleaned up using aids such as brush and dustpan, forceps or cottonwool swabs. Follow the procedure for liquid spills. All aids must be disinfected following use.

1.1.10 Correct Hand Washing Techniques

Wet hands thoroughly and lather with neutral pH soap, vigorously rubbing hands together for at least 10-15 seconds. Wash all parts of the hands, including back, wrists, between fingers and under fingernails. Rinse well under running water. Pat hands dry using disposable paper towel. Do not touch taps with clean hands - if elbows or foot controls are not available, use paper towel to turn the taps off. To minimise irritation and dryness a suitable hand cream may be used.

Bar soap may become contaminated with bacteria if left wet. Liquid soap dispensers are preferred. If liquid soap dispensers are used, they must be cleaned and dried prior to refilling.

1.1.11 Maintenance of Pure Bacteria Stocks

Broth cultures may be stored in the fridge for several weeks.

For short term storage petri dishes (or slopes) are preferred. When cultures are maintained in the short term, a subculture should be plated out before starting a new series of experiments and examined for signs of mixed growth, indicating that the stock has been contaminated. Such contaminated broths should not be used.

The long-term storage of cultures in schools is not recommended.

1.1.12 Microbiology in Home Economics

If microbiology is taught in Home Economics, it may be more appropriate to work in the biology laboratory, particularly once plates are incubated. Swabs taken from food preparation surfaces must not be sub-cultured, and petri dishes should not be opened. Particular care should be taken with surfaces on which poultry have been handled because of the high risk of Salmonella contamination. It must be realised that while heat sterilisation kills microorganisms it does not inactivate toxins that may have already formed in food. Food spoilage should not be studied using meat or meat products because organisms, which cause food poisoning, may be present.

1.1.13 Bibliography

References

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Department of Education and Science, 1985, *Microbiology: An HMI Guide for Schools and Non-advanced Further Education*, Her Majesty's Stationery Office, London.

Standards Australia, AS2243.3-1995 Safety in Laboratories. Part 3. Microbiology.

Standards Australia, AS 4187-1994, Code of practice for cleaning, disinfecting and sterilising reusable medical surgical instruments and equipment, and maintenance of associated environments in health care facilities.

Additional Useful Resources

Australian Academy of Science, 1986, *Web of Life: Student's Manual Part 1*, 10th edit, Australian Academy of Science, Canberra.

Ellis, R. C. & Perry, D. I., 1991, *ABC of Safe Practices for the Biological Science*

Laboratory, Education Resource Centre, Adelaide Medical Centre for Women and Children, Adelaide.

National Health and Medical Research Council, 1996, Infection Control in the Health Care Setting, Commonwealth of Australia, Canberra. http://www.health.gov.au/nhmrc/public/pdf/ic6.pdf (downloaded Aug 1999)

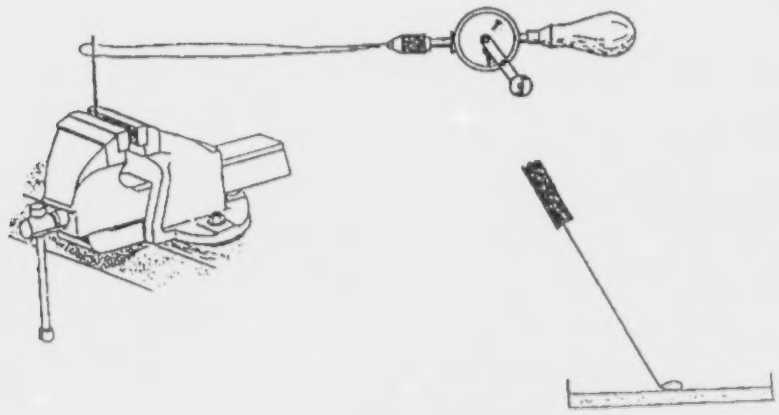
National Health and Medical Research Council, 1999, National Guidelines for Waste Management in the Health Care Industry, Commonwealth of Australia, Canberra. http://www.health.gov.au/nhmrc/public/pdf/eh11.pdf (downloaded Aug 1999)

OSU Environmental Health & Safety Unit, *Oklahoma State University Laboratory Safety Manual,* Oklahoma State University, Stillwater. http://www.pp.okstate.edu/ehs/HAZMAT/LABMAN/HTM (downloaded July 1999)

Secondary Science Curriculum Committee, (no date given), *Techniques in Science,* SA Education Department, Adelaide.

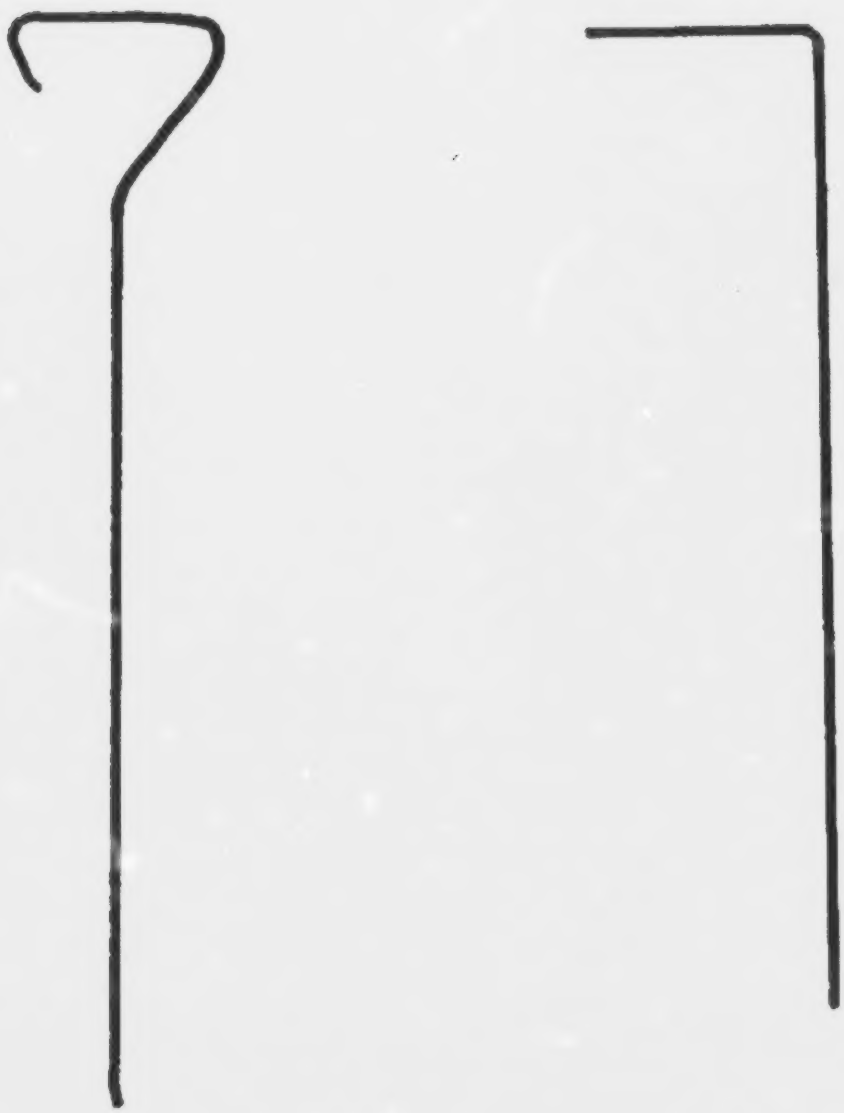
MAKING WIRE LOOPS

Even professional looking loops are easy to make and they do not have loose wire ends to dig into the agar. All you need is a metal vice, a hand drill, a nail (the same diameter ad the diameter of the loop). and some nichrome wire(25G or similar). Put both ends of a 30 cm piece of nichrome wire into the chuck of a hand drill. Secure a nail in the vice, point side up. Put the loop end of the wire over the nail. Now while pulling back wards a little, turn the handle of the drill. Continue turning the drill until the wire breaks where it attaches to the drill. The loop is then simply inserted into a handle (or sealed in glass tubing). It is better if the loop itself is bent upwards to a slight angle, to smoothly skim over the agar.



SPREADER

4mm glass rod



BIOHAZARD

DO NOT STORE FOOD OR DRINK FOR HUMAN CONSUMPTION IN THIS FRIDGE

Standard Operating Procedure for Preparing Nutrient Agar Plates #10 24/11/00

Preparing Nutrient Agar

1. Add 26 g of Chem-Supply Nutrient Agar to a wide necked 2 L conical flask.
2. Add 1 L distilled water. Swirl to mix.
3. Place flask into the aluminum stockpot. Add enough hot water to the stockpot to match the level of solution in the flask.
4. Place the stockpot on the hot plate.
5. Turn the temperature knob to 150 & switch the hotplate on.
6. Heat, with occasional mixing, until the agar solution reaches 95°C, is clear and all the agar powder is dissolved. (This takes >1hr)
7. While wearing heat-proof gloves and while the agar is still hot, pour the solution into 10 X 100 ml Schott bottles. Screw the lid on firmly & then release one quarter turn.
8. Put the Schott bottles onto the wire basket of the autoclave & place the filled basket into the autoclave.
9. Sterilise at 121 °C &15 lb/ in^2 for 20 minutes as describes in SOP #6 Using the Autoclave.
10. Allow the agar to cool to 50-55 °C.*

Pouring Nutrient Agar Plates

1. Prepare the room so that there are no drafts & little pedestrian movement. eg close doors, turn off the airconditioner.
2. Clean the bench with 70% alcohol & paper towel.
3. Lay 40 X 90 mm sterile plastic petri dishes out with the lid side up.
4. Take one bottle of cooled agar and pour 25 ml into a petri dish. Only lifting the lid just enough to allow the agar to be poured & holding the lid over the base while doing so. Repeat with the remaining agar. (4 plates per 100 ml Schott bottle)
5. Allow the agar to set (~30 minutes) before packing upside down (agar side upwards) into a labelled plastic bags & placing them into the fridge.

* Agar will not set above 42°C but only melts at ~95°C.